REMARKS

Applicants respectfully submit that the amendments to the specification and drawings contained herein are made solely in accordance with 37 C.F.R. 1.821 (d), pursuant to the Examiner's request. The amendments submitted herewith do not include new matter. Furthermore, Applicants respectfully submit that all disclosed sequences are properly labeled with SEQ ID Nos., and are included in the Sequence Listing, for which a CRF and paper copy are also submitted herewith.

Prompt and favorable action on this application is respectfully requested. In the event issues remain to be addressed in view of this communication, the Examiner is invited to contact the undersigned by telephone so that a prompt disposition of the present application can be achieved.

Respectfully submitted,

Date June 10, 2002

By _____

FOLEY & LARDNER
P.O. Box 80278
San Diego, California 92138-0278
Telephone: (858) 847-6721
Facsimile: (858) 792-6773

Michael A. Whittaker
Attorney for Applicant
Registration No. 46,230

Appendix A: marked up sheets indicating the changes.

helix formation bind to a broad range of sequences with high affinity and specificity. Although oligonucleotides and their analogs have been shown to interfere with gene expression, the triple helix approach is limited to purine tracks and suffers from poor cellular uptake.

Other small molecules have also been of interest as DNA-binding ligands. Wade, et al. reported the design of peptides that bind in the minor groove of DNA at 5'-(A,T)G(A,T)C(A,T)-3' sequences by a dimeric side-by-side motif (*J. Am. Chem. Soc.* **114**, 8783-8794 (1992)). Mrksich, *et al.* reported antiparallel side-by-side motif for sequence specific-recognition in the minor groove of DNA by the designed peptide 1-methylimidazole-2-carboxamidenetropsin (*Proc. Natl. Acad. Sci. USA* **89**, 7586-7590 (1992)). Pelton, J.G. & Wemmer, D.E. reported the structural characterization of a 2-1 distamycin A-d(CGCAAATTTGGC) (SEQ ID NO: 8) complex by two-dimensional NMR (*Proc. Natl. Acad. Sci. USA* **86**, 5723-5727 (1989)).

Dervan and colleagues have shown that synthetic pyrrole-imidizole polyamides bind DNA with excellent specificity and very high affinities, even exceeding the affinities of many sequence-specific transcription factors (Trauger, et al., *Nature* **382**, 559-561 (1996)). They further describe the recognition of DNA by designed ligands at subnanomolar concentrations. DNA recognition depends on side-by-side amino acid pairing of imidizole-pyrrole or pyrrole-pyrrole pairs in the minor groove. White, S., et al., (1996) reported the effects of the A•T/T•A degeneracy of pyrrole-imidazole polyamide recognition in the minor groove of DNA (*Biochemistry* **35**, 6147-6152 (1996)). White, et al. (1997) reported pairing rules for recognition in the minor groove of DNA by pyrrole-imidazole polyamides (*Chem. & Biol.* **4**, 569-578 (1997)), and demonstrated the 5'-3' N-C orientation preference for polyamide binding in the minor groove. Thus, polyamide molecules thus have the potential to act as inhibitors of protein-DNA interactions in the minor groove.

The development of pairing rules for minor groove binding polyamides derived from N-methylpyrrole (Py) and N-methylimidazole (Im) amino acids provides another means to confer sequence specificity. An Im/Py pair distinguishes G•C from C•G, and both of these from A•T or T•A base pairs, while Py/Im targets a C-G basepair. A Py/Py

the TATA binding protein (TBP). Oligonucleotides corresponding to the HER2/neu TATA box and the adjacent sequences were synthesized. The first oligonucleotide, HERTATA1, has the sequence: (SEQ ID NO: 2)

5'-GCTGCTTGAGGAAGTATAAGAATGAAGTTGTGAAG-3', (the TATA box is in bold). The complementary oligonucleotide, HERTATA2, has the sequence: (SEQ ID NO: 3)

5'-CTTCACAACTTCATTCTTATACTTCCTCAAGCAGC-3'. These complementary 35 base oligonucleotides were 5' end-labeled with γ-^{32}P-ATP and T4 polynucleotide kinase and then annealed to give a double-stranded 35 base pair oligonucleotide. This oligonucleotide was then used in electrophoretic mobility shift assays employing 5% nondenaturing polyacrylamide gels (29:1 acrylamide to bisacrylamide) containing 4 mM $MgCl_2$ and 0.02% (v/v) NP-40 nonionic detergent along with 44 mM Tris-borate, pH 8.3, 1 mM EDTA. The labeled oligo, at a concentration of 0.1 nM, was reacted with 1 nM final concentration of TBP (Promega) in a reaction volume of 20 µl, containing 10% glycerol (v/v), 20 mM HEPES-OH, pH 7.9, 25 mM KCl, 0.025% NP-40 (v/v), 100 µg/ml bovine serum albumin, 0.5 mM dithiothreitol, 0.8 mM spermidine, 0.1 mM EDTA, 2 mM $MgCl_2$.

Various concentrations of the different polyamides, ranging from 0.1 nM to 30 nM, were added to this binding reaction. The reactions were subjected to polyacrylamide gel electrophoresis and the dried gels were subsequently imaged and quantified using a Molecular Dynamics phosphorimager equipped with ImageQuant software. The results are shown in (Figure 4). It is clear that the HER2/neu-specific polyamides (polyamides HER2-1 (Figure 4A), 70 (Figure 4B, squares), and RPR70, of composition ImPyPyPy-γ-PyPyPyPy-β-RPR, (Figure 4B, x's) significantly decrease TBP binding to the HER2/neu TATA box *in vitro*. A control polyamide **86** (Figure 4B, circles), which is not specific for the HER2/neu TATA box, has little effect on the binding of TBP to the HER2/neu TATA box.

"RPR" indicates the presence of a charged arginine-proline-arginine tail on the polyamide.

reflecting the relative levels of HER2/neu mRNA. The PCR primers were: (Her2A) 5'-GCTGGCCCGATGTATTTGATGGT-3' (SEQ ID NO: 4) and (Her2B) 5'-GTTCTCTGCCGTAGGTGTCCCTTT-3' (SEQ ID NO: 5), and 50 ng of each were used in PCR reactions as described below.

The relative amounts of HER2/neu mRNA from the various cells can be determined using reverse transcriptase (RT)-polymerase chain reaction (PCR). After total RNA has been extracted from the polyamide-treated breast cancer cells, as described above, the concentration of total RNA is determined by spectrophotometry (using the optical density at 260 nM) for each different cell type and polyamide concentration. An equal amount of total RNA (10 ng) is used for each RT-PCR. RT-PCR was carried out using the Reverse Transcription System kit (Promega). Using an oligo dT primer, cDNAs are synthesized from the mRNA templates by the enzyme reverse transcriptase at 42° C for 25 min, as recommended. These cDNAs are then used as templates for PCR. Using the buffers and Taq polymerase provided in the kit, PCR was carried out at 26 cycles of denaturation at 94° C for 45 seconds, annealing at 60° C for 45 seconds, and extension at 72° C for 2 minutes. Five μCi of the radioactive nucleotide α-^{32}P-dATP is included in the PCR step to produce a radiolabeled PCR product which can be analyzed on an acrylamide gel and visualized by autoradiography. The relative amount of PCR-product can be quantitated using a Phosphorimager (Molecular Dynamics). The level of HER2/neu mRNA from cells which have not been treated with polyamide are the positive control and are given a value of 1.0 and the HER2/neu mRNA levels for the polyamide-treated samples are given a value relative to the value for untreated cells.

The results of these RT-PCR assays are shown in Figure 6. Treatment of the cell lines SK-BR-3 and Hs 578-T with polyamide HER2-1 for 1-2 days resulted in slightly less than two-fold reduction in the relative levels of HER2/neu mRNA. The control polyamide HIV-1 had no apparent effect on the relative levels of HER2/neu mRNA. When SK-BR-3 cells were treated for 6 days with either polyamide HER2-1 or 70, the relative levels of mRNA decreased more significantly than for the 1-2 day treated cells. SK-BR-3 cells showed a 4-fold and 3-fold decrease in the relative levels of HER2/neu mRNA when treated with polyamide HER2-1 or 70, respectively. These results suggest